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Selected US specifications from IPC sub-class
G01N

(54) Analysis

(57) The relative proportions of glycated and non-glycated haemoglobin in a sample are determined by adding the sample to the first zone (12) of a device comprising a porous solid phase carrier incorporating two distinct zones (12,14). The first is capable of binding only glycated haemoglobin and the second is capable of binding any haemoglobin. The sample is eluted through the device by means of an eluting solvent and haemoglobin that has become bound in either zone is rendered visible by means of a colour-producing reaction which exploits the peroxidatic activity of haemoglobin. The relative intensities of the colour formed in each zone being thereafter used to provide an indication of the relative amounts of haemoglobin in the original sample. Binding of the glycated haemoglobin in the first zone is achieved by boronic acid residues linked to the carrier. The second zone may contain an ion-exchange resin.

Fig. 1

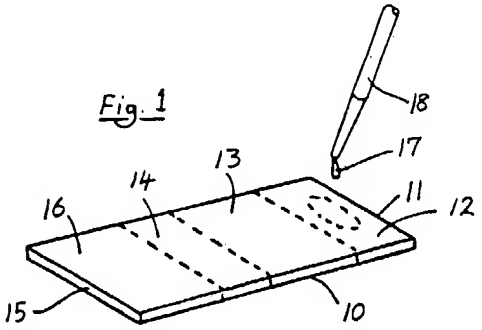
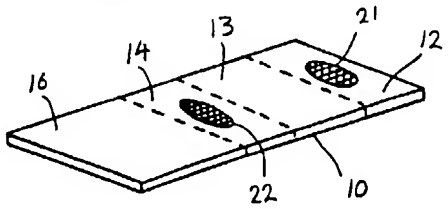


Fig. 4



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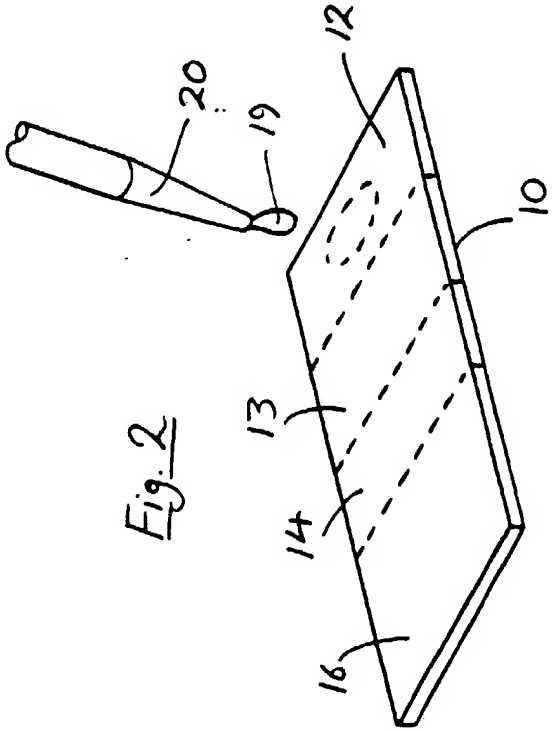

Fig. 2

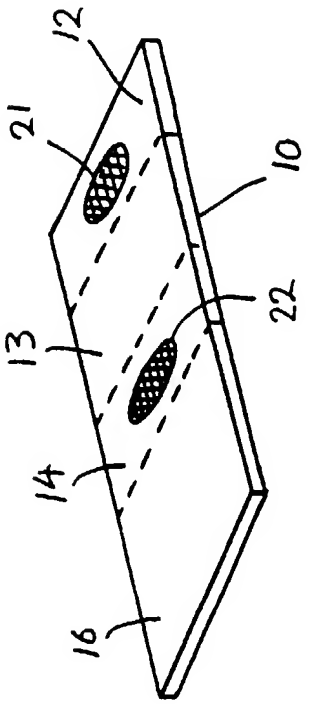

Fig. 4

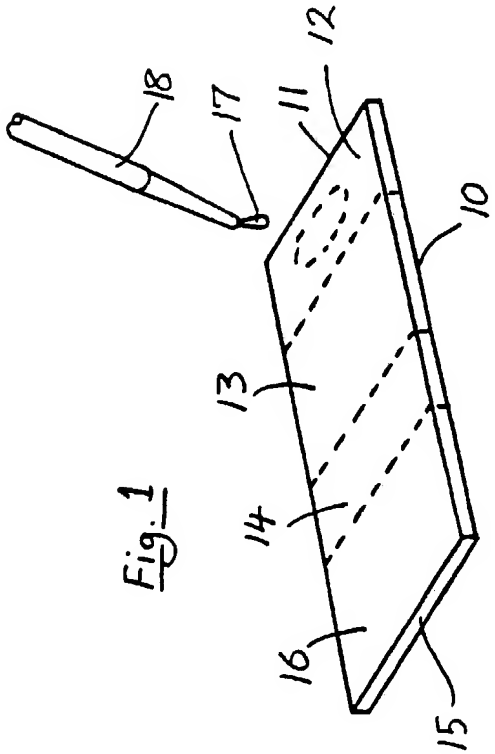

Fig. 1

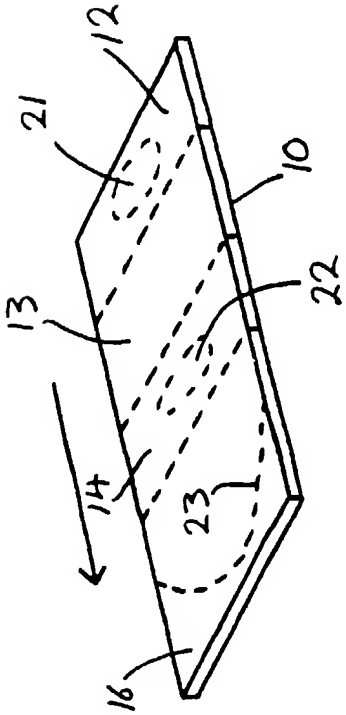

Fig. 3

ANALYSIS

The present invention relates to the analysis of biological samples, and in particular to the analysis of samples such as blood for the level of glycated haemoglobin present therein.

The level of glycated haemoglobin in blood samples has been recognised as indicative of the average blood glucose level over the previous 2-3 months. The relative proportion of glycated to non-glycated haemoglobin in a blood sample is therefore an important indicator of diabetic control.

Techniques for separating and comparing the glycated and non-glycated haemoglobin fractions in blood samples have already been developed. Some techniques are based on the ability of boronic acid to bind glycated haemoglobin. The boronic acid can be linked to a solid phase carrier, for example agarose, and used to extract the glycated

haemoglobin fraction from a sample by column chromatography. Subsequently, the glycated haemoglobin can be released from the boronic acid, e.g. by means of sorbitol. The concentration of the separate glycated and non-glycated haemoglobin fractions can be determined by measurement of absorbance at 415nm.

The present invention provides an improvement over the current analytical techniques which utilise boronic acid as a selective binder for glycated haemoglobin, and enables the separation of glycated and non-glycated haemoglobin and their measurement in situ.

An important embodiment of the invention is an assay device comprising a porous solid phase carrier through which a haemoglobin-containing sample can be eluted, incorporating two distinct zones through which the eluted sample will pass, the first of which zones is capable of binding only glycated haemoglobin and the second of which zones is capable of binding all residual haemoglobin that has passed through the first zone.

The invention includes a procedure for determining the relative proportions of glycated and non-glycated haemoglobin in a sample, wherein the sample is applied to the first zone of a device as set forth in the preceding paragraph, the sample is eluted through the second zone of the device by means of an eluting solvent, and haemoglobin that has become bound in either zone is rendered visible by means of a colour- producing reaction which exploits the peroxidatic activity of haemoglobin, the relative intensities of the colour formed in each zone being thereafter used to provide an indication of the relative amounts of haemoglobin in the original sample.

By the present invention we have found that porous matrices, preferably of a fibrous nature such as paper matrices, can be used as carriers in assays utilising boronic acid as a binding reagent for glycated haemoglobin. The binding ability of such matrices can be enhanced if they are impregnated with water-insoluble polysaccharides having a high level of hydroxyl groups, such as dextran or more preferably agarose, prior to linking of boronic acid to the carrier. This can be achieved, for example, by dipping a paper carrier into molten agarose. Case should be taken to ensure that not so much agarose is applied to the paper that the porous structure of the paper is lost, as this will inhibit the permeability of the paper during use in an assay procedure.

In one embodiment, the invention provides an assay device incorporating, at least in part, an agarose-impregnated solid phase carrier material on which is immobilised boronic acid. The solid phase carrier material can be in any physical shape or form useful in an analytical procedure, such as a strip, sheet, dipstick or particulate material.

In another embodiment of the invention, an assay device incorporates a porous solid phase carrier material having a first binding zone to which a sample suspected of containing glycated haemoglobin can be applied, said first binding zone comprising carrier material on which is immobilised boronic acid, and a second binding zone which is spatially distinct from the first binding zone but linked thereto by the porous carrier material such that a liquid sample can flow from the first binding zone into the second binding zone, the second binding zone comprising carrier material adapted to bind non-glycated haemoglobin.

Preferably the zone binding the glycated haemoglobin is paper. This can be activated, for example by treatment with carbonyl diimidazole, and then immersed in amino-phenyl boronic acid or amino-caproylphenyl boronic acid. The binding capacity can be enhanced, and the non-specific binding can be minimised, by impregnating the paper with agarose prior to activation. Alternatively, the agarose can be activated with carbonyl diimidazole and the boronic acid coupled to the agarose prior to impregnation of the paper.

Ideal papers are made by 3M, and by Schleicher and Schuell (No. 2668).

An ion-exchange resin can be used to provide the required haemoglobin binding ability in the second binding zone. Such resin can be a cation-exchanger, such as phosphate-containing paper, or an anion-exchanger, e.g. containing amino-ethyl groups. Suitable resins are readily available commercially.

A multi-zone assay device of the invention can be fabricated using a single piece of carrier material. However, for ease of manufacture, it can be convenient to prepare the zones as separate portions and to link them in series to provide the complete device. Care should be taken to ensure that the portions are linked together such that eluate can flow easily from each portion to the next.

By way of example only, a preferred embodiment of the invention will now be described with reference to the accompanying drawings.

Example

Figures 1 to 4 of the accompanying drawings depict an assay device in accordance with the invention, and show stages in its use for the purposes of separating haemoglobin into glycated and non-glycated fractions.

Referring to Figure 1, the device comprises an elongate rectangular sheet 10 of carrier material lying flat in a horizontal position. At its one end 11 a first binding zone 12 extends laterally across the entire width of sheet 10. Adjacent the first binding zone 12 is an intermediate zone 13 which separates the first binding zone 12 from a second binding zone 14 which also extends laterally across the entire sheet. Beyond the second binding zone 14 and adjacent the other end 15 of the sheet 10 is an absorbent zone 16 which can take up liquid sample that has migrated from the first binding zone 12 through the sheet via the second zone 14.

The first binding zone 12 is has immobilised on it boronic acid. This zone is therefore capable of selectively binding and retaining glycated haemoglobin present in a sample (e.g. blood haemolysate serum) which is placed on the first binding zone. The second binding zone 14 will contain an ion-exchange resin which is capable of binding all haemoglobin (whether glycated or non-glycated). If the glycated haemoglobin fraction in a sample has previously been absorbed in the first binding zone any haemoglobin which is subsequently absorbed in the second binding zone should be the non-glycated fraction.

In operation, a small quantity 17 of blood haemolysate, containing glycated haemoglobin, is added dropwise by means of a pipette 18 to the first binding zone 12. After allowing appropriate time for the boronic

acid to bind any glycated haemoglobin present in the sample, a wash buffer 19 (e.g. aqueous ammonium acetate at pH 8) is added dropwise by means of pipette 20 to the first binding zone 12 in a quantity sufficient to promote fluid permeation through the test sheet via the intermediate zone 13 and second binding zone 14 and into the terminal absorbent zone 16. As the buffer solution permeates the test strip, the non-glycated fraction of the haemoglobin in the applied sample will be carried from the first binding zone 12 into the second binding zone 14 where it will be retained by the ion exchange resin. Figure 3 represents the device when this transfer has occurred, and the first binding zone 12 contains a region 21 of bound glycated haemoglobin, the second binding zone 14 contains a region 22 of bound non-glycated haemoglobin, and the solvent front 23 has progressed into the absorbent zone 16.

After the elution of the sample has been completed, the zones of bound haemoglobin can be visualised by exploiting the peroxidatic activity of haemoglobin, e.g. by the addition of tetra methyl benzidine to the test strip to create a blue colour in the haemoglobin-containing zones. The relative intensities of the blue colour formed in the first binding zone and the second binding zone can be measured, e.g. by reflectance measurement, and will provide an indication of the relative amounts of glycated haemoglobin and non-glycated haemoglobin in the original blood sample.

The test strip can comprise a single continuous sheet of carrier material or, for ease of manufacture, can be prepared as individual separate zones which are subsequently linked in series so that the liquid sample can flow easily from one end of the composite strip to the other.

If desired, the test strip can also comprise a liquid application zone, in advance of the boronic acid-containing zone 12, to which the test sample and/or the eluting buffer can be applied.

Claims:

1. An assay device comprising a porous solid phase carrier through which a haemoglobin-containing sample can be eluted, incorporating two distinct zones through which the eluted sample will pass, the first of which zones is capable of binding only glycated haemoglobin and the second of which zones is capable of binding all residual haemoglobin that has passed through the first zone.

2. A device according to claim 1, wherein the binding of the glycated haemoglobin in the first zone is achieved by boronic acid residues linked to the carrier.

3. An assay device according to claim 2, wherein the material comprising the first zone is impregnated with a water-insoluble polysaccharide having a high level of hydroxyl groups, prior to the linking of the boronic acid to the carrier.

4. An assay device according to claim 3, wherein the polysaccharide is agarose.

5. An assay device according to any one of the preceding claims, wherein the binding of the residual haemoglobin in the second zone is achieved by an ion-exchange resin in the second zone.

6. A device according to any one of the preceding claims wherein the carrier comprises a single strip or sheet of material.

7. A device according to any one of the preceding claims, wherein the carrier is a fibrous material.

8. A device according to claim 6, wherein the carrier is paper.

9. A procedure for determining the relative proportions of glycated and non-glycated haemoglobin in a sample, wherein the sample is applied to the first zone of a device according to any one of the preceding claims, the sample is eluted through the second zone of the device by means of an eluting solvent, and haemoglobin that has become bound in either zone is rendered visible by means of a colour- producing reaction which exploits the peroxidatic activity of haemoglobin, the relative intensities of the colour formed in each zone being thereafter used to provide an indication of the relative amounts of haemoglobin in the original sample.

10. A method for determining the relative amounts of glycated and non-glycated haemoglobin in a sample, substantially as hereinbefore described in the Example.